PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A23J 3/30 — A1

(11) International Publication Number: WO 92/21248

(43) International Publication Date: 10 December 1992 (10.12.92)

(21) International Application Number: PCT/DK92/00170

(22) International Filing Date: 27 May 1992 (27.05.92)

(30) Priority data:
91610049.8 31 May 1991 (31.05.91) EP
(34) Countries for which the regional or international application was filed: AT et al.

(71) Applicant *(for all designated States except US):* DANMARK PROTEIN A/S [DK/DK]; Nr. Vium, DK-6920 Videbaek (DK).

(72) Inventors; and
(75) Inventors/Applicants *(for US only) :* NIELSEN, Per, Munk [DK/DK]; Rytterstien 29A, DK-3400 Hilleroed (DK). ERIKSEN, Svend [DK/DK]; Delfinvej 8, DK-3450 Alleroed (DK). HANSEN, Ole, Regnar [DK/DK]; Kastagervej 23B, DK-2730 Herlev (DK).

(74) Agent: PEDERSEN, Bent; Danmark Protein A/S, Nr. Vium, DK-6920 Videbaek (DK).

(81) Designated States: AT (European patent), AU, BE (European patent), BR, CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, KR, LU (European patent), MC (European patent), NL (European patent), NO, RU, SE (European patent), US.

Published
With international search report.
With amended claims and statement.

(54) Title: METHOD FOR PRODUCTION OF A WHEY PROTEIN HYDROLYZATE

(57) Abstract

The method for production of a whey protein hydrolyzate comprises the use of a whey protein product with a protein content of at least 65 %, calculated as dry matter as a starting material and a combination of a non-pH-stat hydrolysis followed by an ultrafiltration/microfiltration. The method provides a well tasting and organoleptically acceptable product in high yield.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	ML	Mali
AU	Australia	FR	France	MN	Mongolia
BB	Barbados	GA	Gabon	MR	Mauritania
BE	Belgium	GB	United Kingdom	MW	Malawi
BF	Burkina Faso	GN	Guinea	NL	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IE	Ireland	RO	Romania
CA	Canada	IT	Italy	RU	Russian Federation
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland			SN	Senegal
CI	Côte d'Ivoire	KR	Republic of Korea	SU	Soviet Union
CM	Cameroon	LI	Liechtenstein	TD	Chad
CS	Czechoslovakia	LK	Sri Lanka	TG	Togo
DE	Germany	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		
ES	Spain	MG	Madagascar		

METHOD FOR PRODUCTION OF A WHEY PROTEIN HYDROLYZATE

The invention comprises a method for production of a whey protein hydrolyzate.

Many methods for production of a protein hydrolyzate with good organoleptic properties can be carried out with a low yield only. Thus, it is the purpose of the invention to indicate a method for production of a protein hydrolyzate with good organoleptic properties, which can be carried out with a relatively high yield.

Surprisingly, according to the invention it has been found that a certain combination of a non-pH-stat hydrolysis and an ultrafiltration/microfiltration provides a process for production of a well tasting and organoleptically acceptable product in high yield.

Thus, the method according to the invention for production of a whey protein hydrolyzate is characterized by the fact

1) that a whey protein product with at least 65% protein calculated as dry matter and water is mixed to a slurry with a protein content up to about 20%, preferably up to 12%,
2) that a heat treatment to a temperature above 60°C is carried out,
3) that the mixture from step 2) is proteolytically hydrolyzed by means of at least one protease, by means of a non-pH-stat method to a DH of between 15 and 35%,
4) that the mixture from step 3) is separated on an ultrafiltration/microfiltration unit with cut-off value above 10,000, the permeate constituting the protein hydrolyzate, and
5) that the hydrolysis is terminated by inactivation of the enzyme(s).

It is to be understood that all kinds of whey protein products can be used in step 1, e.g. the usual whey protein produced in relation to cheese manufacture.

It is to be understood that the enzyme inactivation (step 5)) can be carried out before the ultrafiltration/microfiltration (step 4)). Also, it is to be understood that step 5 can be omitted altogether, if the cut-off value of the membrane is low enough to retain all enzymes in the concentrate.

A whey protein hydrolyzate with a composition similar to the whey protein hydrolyzate produced by means of the method according to the invention is described in US 4,427,658.

Also, EP 226221 describes a whey protein hydrolyzate, which however, in contradistinction to the whey protein hydrolyzate produced by means of the method according to the invention is free from lactose and is produced by means of the pH-stat techniques.

Also, US 4,293,571, EP 321603 and EP 322589 describe a whey protein hydrolyzate, which is produced by hydrolysis with subsequent heat treatment, in contradistinction to the whey protein hydrolyzate produced by means of the method according to the invention, i.e. by means of heat treatment with subsequent hydrolysis. The high values of the degree of hydrolysis which can be obtained according to the invention, cannot be obtained with the prior art methods.

EP 65663 describes a whey protein hydrolyzate, which is produced without heat treatment before the hydrolysis, in contradistinction to the method according to the invention.

In Research Disclosure, August 1981 no. 20826 a method similar to the method according to the invention is described. However, the prior art method is restricted to blood as the starting material, and also, the prior art method is performed by means of the pH-stat method.

To the best of the applicant's knowledge, all prior art methods for production of a whey protein hydrolyzate give rise to a whey protein hydrolyzate with an unacceptable taste. The whey protein hydrolyzate according to the invention has a marked agreeable taste. Also, in relation to many of the prior art methods for production of whey protein hydrolyzate the end product is obtained in a low yield and/or at high production cost.

Many of the prior art methods for production of whey protein hydrolyzate give rise to a whey protein hydrolyzate which is not heat stable and not fully soluble in a broad pH interval. The whey protein hydrolyzate produced by means of the method according to the invention is heat stable and fully soluble in a broad pH interval.

A preferred embodiment of the method according to the invention comprises that the slurry in step 1) has a protein content of 7-12%. In this manner the equipment is utilized optimally, and also, the viscosity is not too high for handling.

A preferred embodiment of the method according to the invention comprises that the heat treatment in step 2) is carried out between 70 and 90°C. This temperature interval is especially well suited in relation to performance of the usually used heat exchangers.

A preferred embodiment of the method according to the invention comprises that the pH adjustment in step 3) is carried out by means of $Ca(OH)_2$ and/or KOH. In this manner a better taste is obtained, and also, a favorable mineral distribution in the final product is obtained. Also, sodium carbonate or sodium phosphate can be used for pH adjustment in order to precipitate the Ca^{++} in the raw whey protein product.

A preferred embodiment of the method according to the invention comprises that the hydrolysis in step 3) is carried out to a DH of between 20-30. In this manner a product with excellent organoleptic properties is obtained.

A preferred embodiment of the method according to the invention comprises that a protease producible by means of *B. licheniformis*, preferably Alcalase®, and/or a protease producible by means of *B. subtilis*, preferably Neutrase®, and/or trypsin is used as proteolytic enzyme(s). It is especially preferred to use Alcalase® (with a high pH optimum) first, and then Neutrase® (with a lower pH optimum). This method is especially well suited to the non-pH-stat-method used according to the inv ntion.

A preferred embodiment of the method according to the invention comprises that the cut-off value of the ultrafiltration/microfiltration unit is above 50.000. In this manner a very high flux is obtainable.

A preferred embodiment of the method according to the invention comprises that the inactivation of the enzyme(s) (step 5)) is carried out by heat treatment. This inactivation is especially well suited in case the pH of the final protein hydrolyzate is supposed to be relatively high (around neutrality).

A preferred embodiment of the method according to the invention comprises that the inactivation of the enzyme(s) (step 5)) is carried out by acid treatment. This inactivation is especially well suited in case the pH of the final protein hydrolyzate is supposed to be relatively low (acidic).

A preferred embodiment of the method according to the invention comprises that the mixture at the end of step 4) is treated with activated carbon for more than 5 minutes at a temperature, which is preferably between 50 and 70°C in an amount corresponding to between 1 and 5% carbon, calculated in relation to dry matter content, and that the activated carbon is removed. In this manner the flavor is improved.

A preferred embodiment of the method according to the invention comprises that after step 5) a concentration is carried out by nanofiltration/hyperfiltration/reverse osmosis at a temperature, which is preferably between 50 and 70°C and/or evaporation, whereafter the retentate is collected as the protein hydrolyzate solution. By means of the nanofiltration a desalination can be carried out by proper selection of the membrane; besides nanofiltration/hyperfiltration/reverse osmosis is an inexpensive way for removal of water. Evaporation has the advantage of obtaining a high dry matter content in the concentrate before drying.

A preferred embodiment of the method according to the invention comprises that the protein hydrolyzate solution from step 5) is spray-dried to a water content below 6.5%. In this manner a stable product is obtained, both microbially and organol ptically.

The method according to the invention will be illustrated in the following examples.

For the sake of a better overview a survey of some of the parameters which are changed in the examples appear from the table shown below.

Example no.	Inactivation H = heat A = acid	UF modul	pH N = neutral A = acid	pH adjustment Ca= $Ca(OH)_2$ Na = NaOH	Carbon treatment
1	H	PCI	N	Ca	+
2	H	PCI	N	Na	+
3	A	PCI	A	Ca	+
4	H	DDS	N	Ca	+
5	H	PCI	N	Ca	÷
6	H	PCI	N	Ca	+
7	H	PCI	N	Ca	+
8	A	DDS	A	Na	+

EXAMPLE 1

Feed

The starting material is spray-dried whey protein concentrate with approx. 80% protein calculated as dry matter.

Mixing

The raw material is diluted with deionized water to a protein content of 8%. The optimum temperature for fast solubilisation of the protein is 55-60°C.

Heat treatment

Pasteurisation is carried out in a heat exchanger for at least 2 minutes at 85°C. The purpose is to denature the protein in order to make the hydrolysis more

efficient. Also in this manner a very low bacterial count before the incubation with the enzymes is obtained.

pH adjustment

pH is adjusted to 8.0 with $Ca(OH)_2$. About 1% of $Ca(OH)_2$ based on amount of protein is needed.

Hydrolysis

Temperature 53-54°C.

Enzyme 1: Alcalase® 2.4 L. Dosage E/S = 2.2%

Enzyme 2: Neutrase® 0.5 L. Dosage E/S = 1.1%. Neutrase is added when the pH has decreased to < 7.0.

Process time 12 hours. The enzymatic hydrolysis is monitored by the osmolality. The increase in osmolality should be 175 mOsm/kg (measured with a concentration of 8% of protein in the slurry).

Ultrafiltration separation

The UF-plant used comprises PCI modules mounted with FP100 membranes with cut-off value 100,000.

Concentration to half of the initial volume and subsequent diafiltration with twice the volume of the concentrate. Final concentration to maximum dry matter content.

Temperature 60-65°C.

The retentate which is the main by-product of the process is discharged.

Inactivation

The permeate is heat treated for 3 minutes at 85°C in order to inactivate the enzymes and for bacteriological reasons.

Nanofiltration

Concentration to 25-30° Brix

Temperature 55-60°C

The nanofiltration permeate appearing as a by-product is discharged

Treatment with activated carbon

4% activated carbon (Picatif FGV 120) based on amount of dry matter measured as °Brix is added to the nanofiltration retentate at 55-60°C. Reaction time 30 minutes.

Filtration

Removal of activated carbon on plate filter

Final products

The whey protein hydrolyzate concentrate with a dry matter content of 25% is further processed by sterile filtration and spray-drying, the spray-drying being performed by drying the whey protein hydrolyzate concentrate at T_i = 200°C and T_o = 75°C in a spray-dryer with atomization wheel.

Characterization of the whey protein hydrolyzate concentrate obtained:

Taste:	No off-flavor and a low degree of bitterness	
Composition:	Dry matter	95%
	Protein in dry matter (N*6.38)	89.5%
	Ash in dry matter	4.4%
	Fat in dry matter	<0.1%
Properties:	Solubility	Fully soluble
	pH in solution with 5% protein	5.9
	Osmolality in solution with 5% protein	< 200 mOsm/kg

Molecular distribution:	Degree of hydrolysis	24.8%
	Mw	1030
	Mn	500
	Average peptide chain length	4.0

EXAMPLE 2

Feed

The starting material is spray-dried whey protein concentrate with approx. 80% protein calculated as dry matter.

Mixing

The raw material is diluted with deionized water to a protein content of 8%. The optimum temperature for fast solubilisation of the protein is 55-60°C.

Heat treatment

Pasteurisation is carried out in a heat exchanger for at least 2 minutes at 85°C. The purpose is to denature the protein in order to make the hydrolysis more efficient. Also in this manner a very low bacterial count before the incubation with the enzymes is obtained.

pH adjustment

pH is adjusted to 8.0 with 4N NaOH.

Hydrolysis

Temperature 53-54°C.

Enzyme 1: Alcalase® 2.4 L. Dosage E/S = 2.2%

Enzyme 2: Neutrase 0.5 L. Dosage E/S = 1.1%. Neutrase is added when the pH has decreased to < 7.0.

Process time 12 hours. The enzymatic hydrolysis is monitored by the osmolality. The increase in osmolality should be 175 mOsm/kg (measured with a concentration of 8% of protein in the slurry).

Ultrafiltration separation

The UF-plant used comprises PCI modules mounted with FP100 membranes with cut-off value 100,000.

Concentration to half of the initial volume and subsequent diafiltration with twice the volume of the concentrate. Final concentration to maximum dry matter content.

Temperature 60-65°C.

The retentate which is the main by-product of the process is discharged.

Inactivation

The permeate is heat treated for 3 minutes at 85°C in order to inactivate the enzymes and for bacteriological reasons.

Nanofiltration

Concentration to 25-30° Brix

Temperature 55-60°C

The nanofiltration permeate appearing as a by-product is discharged

Treatment with activated carbon

4% activated carbon (Picatif FGV 120) based on amount of dry matter measured as °Brix is added to the nanofiltration retentate at 55-60°C. Reaction time 30 minutes.

Filtration

Removal of activated carbon on plate filter

Final products

The whey protein hydrolyzate concentrate with a dry matter content of 25% is further processed by sterile filtration and spray-drying, the spray-drying being performed by drying the whey protein hydrolyzate concentrate at T_i = 200°C and T_o = 75°C in a spray-dryer with atomization wheel.

Characterization of the whey protein hydrolyzate concentrate obtained:

Taste:	No off-flavor and a low degree of bitterness	
Composition:	Dry matter	94.5%
	Protein in dry matter (N*6.38)	84%
	Ash in dry matter	4%
	Fat in dry matter	<0.1%
Properties:	Solubility	Fully soluble
	pH in solution with 5% protein	6.5
	Osmolality in solution with 5% protein	< 200 mOsm/kg
Molecular distribution:	Degree of hydrolysis	27%
	Mw	800
	Mn	400
	Average peptide chain length	3.5

EXAMPLE 3

Feed

The starting material is spray-dried whey protein concentrate with approx. 80% protein calculated as dry matter.

Mixing

The raw material is diluted with deionized water to a protein content of 8%. The optimum temperature for fast solubilisation of the protein is 55-60°C.

Heat treatment

Pasteurisation is carried out in a heat exchanger for at least 2 minutes at 85°C. The purpose is to denature the protein in order to make the hydrolysis more efficient. Also in this manner a very low bacterial count before the incubation with the enzymes is obtained.

pH adjustment

pH is adjusted to 8.0 with $Ca(OH)_2$. About 1% of $Ca(OH)_2$ based on amount of protein is needed.

Hydrolysis

Temperature 53-54°C.

Enzyme 1: Alcalase® 2.4 L. Dosage E/S = 2.2%

Enzyme 2: Neutrase® 0.5 L. Dosage E/S = 1.1%. Neutrase is added when the pH has decreased to < 7.0.

Process time 12 hours. The enzymatic hydrolysis is monitored by the osmolality. The increase in osmolality should be 175 mOsm/kg (measured with a concentration of 8% of protein in the slurry).

pH-adjustment

pH is adjusted to 4.2 by means of 30% HCl in order to obtain an end product suitable for fortifying acidic beverages with protein.

Ultrafiltration separation

The UF-plant used comprises PCI modules mounted with FP100 membranes with cut-off value 100,000.

Concentration to half of the initial volume and subsequent diafiltration with twice the volume of the concentrate. Final concentration to maximum dry matter content.

Temperature 60-65°C.

The retentate which is the main by-product of the process is discharged.

Pasteurization

The permeate is heat treated for 30 seconds at 75°C for bacteriological reasons.

Nanofiltration

Concentration to 25-30° Brix

Temperature 55-60°C

The nanofiltration permeate appearing as a by-product is discharged.

Treatment with activated carbon

4% activated carbon (Picatif FGV 120) based on amount of dry matter measured as °Brix is added to the nanofiltration retentate at 55-60°C. Reaction time 30 minutes.

Filtration

Removal of activated carbon on plate filter

Final products

The whey protein hydrolyzate concentrate with a dry matter content of 25% is further processed by sterile filtration and spray-drying, the spray-drying being performed by drying the whey protein hydrolyzate concentrate at T_i = 200°C and T_o = 75°C in a spray-dryer with atomization wheel.

Characterization of the whey protein hydrolyzate concentrate obtained:

Taste:	No off-flavor and a low degree of bitterness	
Composition:	Dry matter	94.5%
	Protein in dry matter (N*6.38)	84%
	Ash in dry matter	4%
	Fat in dry matter	<0.1%
Properties:	Solubility	Fully soluble
	pH in solution with 5% protein	4.2
	Osmolality in solution with 5% protein	< 200 mOsm/kg
Molecular distribution:	Degree of hydrolysis	27%
	Mw	800
	Mn	400
	Average peptide chain length	3.5

EXAMPLE 4

Feed

The starting material is spray-dried whey protein concentrate with approx. 80% protein calculated as dry matter.

Mixing

The raw material is diluted with deionized water to a protein content of 8%. The optimum temperature for fast solubilisation of the protein is 55-60°C.

Heat treatment

Pasteurisation is carried out in a heat exchanger for at least 2 minutes at 85°C. The purpose is to denature the protein in order to make the hydrolysis more efficient. Also in this manner a very low bacterial count before the incubation with the enzymes is obtained.

pH adjustment

pH is adjusted to 8.0 with $Ca(OH)_2$. About 1% of $Ca(OH)_2$ based on amount of protein is needed.

Hydrolysis

Temperature 53-54°C.

Enzyme 1: Alcalase® 2.4 L. Dosage E/S = 2.2%

Enzyme 2: Neutrase® 0.5 L. Dosage E/S = 1.1%. Neutrase is added when the pH has decreased to < 7.0.

Process time 12 hours. The enzymatic hydrolysis is monitored by the osmolality. The increase in osmolality should be 175 mOsm/kg (measured with a concentration of 8% of protein in the slurry).

Ultrafiltration separation

The UF-plant used comprises DDS modules mounted with GR40PP membranes with cut-off value 100,000.

Concentration to half of the initial volume and subsequent diafiltration with twice the volume of the concentrate. Final concentration to maximum dry matter content.

Temperature 60-65°C.

The retentate which is the main by-product of the process is discharged.

Inactivation

The permeate is heat treated for 3 minutes at 85°C in order to inactivate the enzymes and for bacteriological reasons.

Nanofiltration

Concentration to 25-30° Brix

Temperature 55-60°C

The nanofiltration permeate appearing as a by-product is discharged

Treatment with activated carbon

4% activated carbon (Picatif FGV 120) based on amount of dry matter measured as °Brix is added to the nanofiltration retentate at 55-60°C. Reaction time 30 minutes.

Filtration

Removal of activated carbon on plate filter.

Final products

The whey protein hydrolyzate concentrate with a dry matter content of 25% is further processed by sterile filtration and spray-drying, the spray-drying being performed by drying the whey protein hydrolyzate concentrate at T_i = 200°C and T_o = 75°C in a spray-dryer with atomization wheel.

Characterization of the whey protein hydrolyzate concentrate obtained:

Taste:	No off-flavor and a low degree of bitterness	
Composition:	Dry matter	94.5%
	Protein in dry matter (N*6.38)	84%
	Ash in dry matter	4%
	Fat in dry matter	<0.1%

Properties:	Solubility	Fully soluble
	pH in solution with 5% protein	6.5
	Osmolality in solution with 5% protein	< 200 mOsm/kg
Molecular distribution:	Degree of hydrolysis	27%
	Mw	800
	Mn	400
	Average peptide chain length	3.5

EXAMPLE 5

Feed

The starting material is spray-dried whey protein concentrate with approx. 80% protein calculated as dry matter.

Mixing

The raw material is diluted with deionized water to a protein content of 8%. The optimum temperature for fast solubilisation of the protein is 55-60°C.

Heat treatment

Pasteurisation is carried out in a heat exchanger for at least 2 minutes at 85°C. The purpose is to denature the protein in order to make the hydrolysis more efficient. Also in this manner a very low bacterial count before the incubation with the enzymes is obtained.

pH adjustment

pH is adjusted to 8.0 with $Ca(OH)_2$. About 1% of $Ca(OH)_2$ based on amount of protein is needed.

Hydrolysis

Temperature 53-54°C.

Enzyme 1: Alcalase® 2.4 L. Dosage E/S = 2.2%

Enzyme 2: Neutrase® 0.5 L. Dosage E/S = 1.1%. Neutrase is added when the pH has decreased to < 7.0.

Process time 12 hours. The enzymatic hydrolysis is monitored by the osmolality. The increase in osmolality should be 175 mOsm/kg (measured with a concentration of 8% of protein in the slurry).

Ultrafiltration separation

The UF-plant used comprises PCI modules mounted with FP100 membranes with cut-off value 100,000.

Concentration to half of the initial volume and subsequent diafiltration with twice the volume of the concentrate. Final concentration to maximum dry matter content.

Temperature 60-65°C.

The retentate which is the main by-product of the process is discharged.

Inactivation

The permeate is heat treated for 3 minutes at 85°C in order to inactivate the enzymes and for bacteriological reasons.

Nanofiltration

Concentration to 25-30° Brix

Temperature 55-60°C

The nanofiltration permeate appearing as a by-product is discharged

Final products

The whey protein hydrolyzate concentrate with a dry matter content of 25% is further processed by sterile filtration and spray-drying, the spray-drying being performed by drying the whey protein hydrolyzate concentrate at T_i = 200°C and T_o = 75°C in a spray-dryer with atomization wheel.

Characterization of the whey protein hydrolyzate concentrate obtained:

Taste:	Slight off-flavor and a low degree of bitterness	
Composition:	Dry matter	94.5%
	Protein in dry matter (N*6.38)	84%
	Ash in dry matter	4%
	Fat in dry matter	<0.1%
Properties:	Solubility	Fully soluble
	pH in solution with 5% protein	6.5
	Osmolality in solution with 5% protein	< 200 mOsm/kg
Molecular distribution:	Degree of hydrolysis	27%
	Mw	800
	Mn	400
	Average peptide chain length	3.5

EXAMPLE 6

Feed

The starting material is spray-dried whey protein concentrate with approx. 80% protein calculated as dry matter.

Mixing

The raw material is diluted with deionized water to a protein content of 8%. The optimum temperature for fast solubilisation of the protein is 55-60°C.

Heat treatment

Pasteurisation is carried out in a heat exchanger for at least 2 minutes at 85°C. The purpose is to denature the protein in order to make the hydrolysis more efficient. Also in this manner a very low bacterial count before the incubation with the enzymes is obtained.

pH adjustment

pH is adjusted to 8.0 with $Ca(OH)_2$. About 1% of $Ca(OH)_2$ based on amount of protein is needed.

Hydrolysis

Temperature 53-54°C.

Enzyme 1: Alcalase® 2.4 L. Dosage E/S = 2.2%

Enzyme 2: Neutrase® 0.5 L. Dosage E/S = 1.1%. Neutrase is added when the pH has decreased to < 7.0.

Process time 12 hours. The enzymatic hydrolysis is monitored by the osmolality. The increase in osmolality should be 175 mOsm/kg (measured with a concentration of 8% of protein in the slurry).

Treatment with activated carbon

4% activated carbon (Picatif FGV 120) based on amount of dry matter measured as °Brix is added to the mixture at 55-60°C. Ultrafiltration is carried out with activated carbon in the retentate.

Ultrafiltration separation

The UF-plant used comprises PCI modules mounted with FP100 membranes with cut-off value 100,000.

Concentration to half of the initial volume and subsequent diafiltration with twice the volume of the concentrate. Final concentration to maximum dry matter content.

Temperature 60-65°C.

The retentate containing the activated carbon is discharged.

Inactivation

The permeate is heat treated for 3 minutes at 85°C in order to inactivate the enzymes and for bacteriological reasons.

Nanofiltration

Concentration to 25-30° Brix

Temperature 55-60°C

The nanofiltration permeate appearing as a by-product is discharged

Final products

The whey protein hydrolyzate concentrate with a dry matter content of 25% is further processed by sterile filtration and spray-drying, the spray-drying being performed by drying the whey protein hydrolyzate concentrate at T_i = 200°C and T_o = 75°C in a spray-dryer with atomization wheel.

Characterization of the whey protein hydrolyzate concentrate obtained:

Taste:	No off-flavor and a low degree of bitterness	
Composition:	Dry matter	94.5%
	Protein in dry matter (N*6.38)	84%
	Ash in dry matter	4%
	Fat in dry matter	<0.1%
Properties:	Solubility	Fully soluble
	pH in solution with 5% protein	6.5
	Osmolality in solution with 5% protein	< 200 mOsm/kg
Molecular distribution:	Degree of hydrolysis	27%
	Mw	800
	Mn	400
	Average peptide chain length	3.5

EXAMPLE 7

Feed

The starting material is liquid concentrated whey protein with approx. 80% protein calculated as dry matter, produced by ultrafiltration and diafiltration of whey until the wanted protein content, calculated as dry matter

Mixing

The raw material is diluted with deionized wat r to a protein content of 8%. The optimum temperature for fast solubilisation of the protein is 55-60°C.

Heat treatment

Pasteurisation is carried out in a heat exchanger for at least 2 minutes at 85°C. The purpose is to denature the protein in order to make the hydrolysis more efficient. Also in this manner a very low bacterial count before the incubation with the enzymes is obtained.

pH adjustment

pH is adjusted to 8.0 with $Ca(OH)_2$. About 1% of $Ca(OH)_2$ based on amount of protein is needed.

Hydrolysis

Temperature 53-54°C.

Enzyme 1: Alcalase® 2.4 L. Dosage E/S = 2.2%

Enzyme 2: Neutrase® 0.5 L. Dosage E/S = 1.1%. Neutrase is added when the pH has decreased to < 7.0.

Process time 12 hours. The enzymatic hydrolysis is monitored by the osmolality. The increase in osmolality should be 175 mOsm/kg (measured with a concentration of 8% of protein in the slurry).

Ultrafiltration separation

The UF-plant used comprises PCI modules mounted with FP100 membranes with cut-off value 100,000.

Concentration to half of the initial volume and subsequent diafiltration with twice the volume of the concentrate. Final concentration to maximum dry matter content.

Temperature 60-65°C.

The retentate which is the main by-product of the process is discharged.

Inactivation

The permeate is heat treated for 3 minutes at 85°C in order to inactivate the enzymes and for bacteriological reasons.

Nanofiltration

Concentration to 25-30° Brix

Temperature 55-60°C

The nanofiltration permeate appearing as a by-product is discharged.

Treatment with activated carbon

4% activated carbon (Picatif FGV 120) based on amount of dry matter measured as °Brix is added to the nanofiltration retentate at 55-60°C. Reaction time 30 minutes.

Filtration

Removal of activated carbon on plate filter

Final products

The whey protein hydrolyzate concentrate with a dry matter content of 25% is further processed by sterile filtration and spray-drying, the spray-drying being performed by drying the whey protein hydrolyzate concentrate at T_i = 200°C and T_o = 75°C in a spray-dryer with atomization wheel.

Characterization of the whey protein hydrolyzate concentrate obtained:

Taste:	No off-flavor and a low degree of bitterness	
Composition:	Dry matter	94.5%
	Protein in dry matter (N*6.38)	84%
	Ash in dry matter	4%
	Fat in dry matter	<0.1%

Properties:	Solubility	Fully soluble
	pH in solution with 5% protein	6.5
	Osmolality in solution with 5% protein	< 200 mOsm/kg
Molecular distribution:	Degree of hydrolysis	27%
	Mw	800
	Mn	400
	Average peptide chain length	3.5

EXAMPLE 8

Feed

The starting material is spray-dried whey protein concentrate with approx. 80% protein calculated as dry matter.

Mixing

The raw material is diluted with deionized water to a protein content of 8%. The optimum temperature for fast solubilisation of the protein is 55-60°C.

Heat treatment

Pasteurisation is carried out in a heat exchanger for at least 2 minutes at 85°C. The purpose is to denature the protein in order to make the hydrolysis more efficient. Also in this manner a very low bacterial count before the incubation with the enzymes is obtained.

pH adjustment

pH is adjusted to 8.0 with 4N NaOH.

Hydrolysis

Temperature 55°C.

Enzyme 1: Alcalase® 2.4 L. Dosage E/S = 2.0%

Enzyme 2: Trypsin PTN 3.3G. Dosage E/S = 3.0%. Trypsin is added when the DH has reached 16% (after 3 hours and 30 minutes).

Total hydrolysis time: 5 hours and 15 minutes. The enzymatic hydrolysis is monitored by the osmolality.

pH-adjustment

The pH value is adjusted to 4.2 by means of 30% HCl in order to obtain an end product suitable for fortifying acidic beverages with protein.

Ultrafiltration separation

The UF-plant used comprises DDS modules mounted with GR40PP membranes with cut-off value 100,000.

Concentration to half of the initial volume and subsequent diafiltration with twice the volume of the concentrate. Final concentration to maximum dry matter content.

Temperature 60-65°C.

The retentate which is the main by-product of the process is discharged.

Pasteurization

The permeate is heat treated for 30 seconds at 75°C in order to inactivate the enzymes and for bacteriological reasons.

Nanofiltration

Concentration to 25-30° Brix

Temperature 55-60°C

The nanofiltration permeate appearing as a by-product is discharged

Treatment with activated carbon

4% activated carbon (Picatif FGV 120) based on amount of dry matter measured as °Brix is added to the nanofiltration retentate at 55-60°C. Reaction time 30 minutes.

Filtration

Removal of activated carbon on plate filter.

Final products

The whey protein hydrolyzate concentrate with a dry matter content of 25% is further processed by sterile filtration and spray-drying, the spray-drying being performed by drying the whey protein hydrolyzate concentrate at T_i = 200°C and T_o = 75°C in a spray-dryer with atomization wheel.

Characterization of the whey protein hydrolyzate concentrate obtained:

Taste:	No off-flavor and a low degree of bitterness	
Composition:	Dry matter	94.5%
	Protein in dry matter (N*6.38)	85%
	Ash in dry matter	4%
	Fat in dry matter	<0.1%
Properties:	Solubility	Fully soluble
	pH in solution with 5% protein	4.2
	Osmolality in solution with 5% protein	< 200 mOsm/kg
Molecular distribution:	Degree of hydrolysis	21%
	Mw	800
	Mn	595
	Average peptide chain length	4.8

CLAIMS

1. Method for production of a whey protein hydrolyzate, characterized by the fact

1) that a whey protein product with at least 65% protein calculated as dry matter and water is mixed to a slurry with a protein content up to about 20%, preferably up to 12%,

2) that a heat treatment to a temperature above 60°C is carried out,

3) that the mixture from step 2) is proteolytically hydrolyzed by means of at least one protease, by means of a non-pH-stat method to a DH of between 15 and 35%,

4) that the mixture from step 3) is separated on an ultrafiltration/microfiltration unit with cut-off value above 10,000, the permeate constituting the protein hydrolyzate, and

5) that the hydrolysis is terminated by inactivation of the enzyme(s).

2. Method according to Claim 1, characterized by the fact that the slurry in step 1) has a protein content of 7-12%.

3. Method according to Claims 1 - 2, characterized by the fact that the heat treatment in step 2) is carried out between 70 and 90°C.

4. Method according to Claims 1 - 3, characterized by the fact that the pH adjustment in step 3) is carried out by means of $Ca(OH)_2$ and/or KOH.

5. Method according to Claims 1 - 4, characterized by the fact that the hydrolysis in step 3) is carried out to a DH of between 20-30%.

6. Method according to Claims 1 - 5, characterized by the fact that a protease producible by means of *B. licheniformis*, preferably Alcalase®, and/or a protease producible by means of *B. subtilis*, preferably Neutrase®, and/or trypsin is used as proteolytic enzyme(s).

7. Method according to Claims 1 - 6, characterized by the fact that the cut-off value of the ultrafiltration/microfiltration unit is above 50.000.

8. Method according to Claims 1 - 7, characterized by the fact that the inactivation of the enzyme(s) (step 5)) is carried out by heat treatment.

9. Method according to Claims 1 - 7, characterized by the fact that the inactivation of the enzyme(s) (step 5)) is carried out by acid treatment.

10. Method according to Claims 1 - 9, characterized by the fact that the mixture at the end of step 3) or step 5) is treated with activated carbon for more than 5 minutes at a temperature, which is preferably between 50 and 70°C in an amount corresponding to between 1 and 5% carbon, calculated in relation to dry matter content, and that the activated carbon is removed.

11. Method according to Claims 1 - 10, characterized by the fact that after step 5) a concentration is carried out by nanofiltration/hyperfiltration/reverse osmosis at a temperature, which is preferably between 50 and 70°C and/or evaporation, whereafter the retentate is collected as the protein hydrolyzate solution.

12. Method according to Claims 1 - 11, characterized by the fact that the protein hydrolyzate solution from step 5) is spray-dried to a water content below 6.5%.

AMENDED CLAIMS
[received by the International Bureau on 23 October 1992 (23.10.92); original claim 6 deleted; original claims 1 and 7-12 replaced by amended claims 1 and 6-11 (2 pages)]

1. Method for production of a whey protein hydrolyzate, characterized by the fact

1) that a whey protein product with at least 65% protein calculated as dry matter and water is mixed to a slurry with a protein content up to about 20%, preferably up to 12%,

2) that a heat treatment to a temperature above 60°C is carried out,

3) that the mixture from step 2) is proteolytically hydrolyzed by means of a protease producible by means of *B. licheniformis*, preferably Alcalase®, and/or a protease producible by means of *B. subtilis*, preferably Neutrase®, and/or trypsin, by means of a non-pH-stat method to a DH of between 15 and 35%,

4) that the mixture from step 3) is separated on an ultrafiltration/microfiltration unit with cut-off value above 10,000, the permeate constituting the protein hydrolyzate, and

5) that the hydrolysis is terminated by inactivation of the enzyme(s).

2. Method according to Claim 1, characterized by the fact that the slurry in step 1) has a protein content of 7-12%.

3. Method according to Claims 1 - 2, characterized by the fact that the heat treatment in step 2) is carried out between 70 and 90°C.

4. Method according to Claims 1 - 3, characterized by the fact that the pH adjustment in step 3) is carried out by means of $Ca(OH)_2$ and/or KOH.

5. Method according to Claims 1 - 4, characterized by the fact that the hydrolysis in step 3) is carried out to a DH of between 20-30%.

6. Method according to Claims 1 - 5, characterized by the fact that the cut-off value of the ultrafiltration/microfiltration unit is above 50.000.

7. Method according to Claims 1 - 6, characterized by the fact that the inactivation of the enzyme(s) (step 5)) is carried out by heat treatment.

8. Method according to Claims 1 - 6, characterized by the fact that the inactivation of the enzyme(s) (step 5)) is carried out by acid treatment.

9. Method according to Claims 1 - 8, characterized by the fact that the mixture at the end of step 3) or step 5) is treated with activated carbon for more than 5 minutes at a temperature, which is preferably between 50 and 70°C in an amount corresponding to between 1 and 5% carbon, calculated in relation to dry matter content, and that the activated carbon is removed.

10. Method according to Claims 1 - 9, characterized by the fact that after step 5) a concentration is carried out by nanofiltration/hyperfiltration/reverse osmosis at a temperature, which is preferably between 50 and 70°C and/or evaporation, whereafter the retentate is collected as the protein hydrolyzate solution.

11. Method according to Claims 1 - 10, characterized by the fact that the protein hydrolyzate solution from step 5) is spray-dried to a water content below 6.5%.

STATEMENT UNDER ARTICLE 19

The enclosed new set of claims has been amended by incorporation of Claim 6 into the main claim. In this way we have generated a better distinction between the invention and the cited references.

INTERNATIONAL SEARCH REPORT

International Application No PCT/DK 92/00170

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A 23 J 3/30

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	A 23 J

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
P,X	WO, A1, 9110369 (TESSENDERLO CHEMIE N.V.) 25 July 1991, see claim 1, page 9, lines 3-4, lines 29-35 --	1-12
X	US, A, 4427658 (JEAN-LOUIS MAUBOIS ET AL.) 24 January 1984, see col. 7, lines 30-55, col. 13, lines 6-26 --	1-12
Y	EP, A1, 0274946 (LABORATOIRE ROGER BELLON) 20 July 1988, see abstract --	1-12
Y	EP, A1, 0065663 (MILES LABORATORIES INC.) 1 December 1982, see claim 1, 5-6, page 9, lines 1-5 --	1-12

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
26th August 1992	1992 -09- 07
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Kerstin Boije Jansson

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	EP, A1, 0322589 (SOCIETE DES PRODUITS NESTLE S.A.) 5 July 1989, see claim 1 --	1-12
Y	EP, A1, 0226221 (ERNST-GUNNAR SAMUELSSON) 24 June 1987, see col. 8, lines 24-26 --	1-12

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers....., because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the the claims. It is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additonal fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional seach fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/DK 92/00170

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the Swedish Patent Office EDP file on 31/07/92
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A1- 9110369	91-07-25	BE-A-	1003298	92-02-18
US-A- 4427658	84-01-24	AU-B-	535601	84-03-29
		AU-D-	5966780	81-01-08
		CA-A-	1150564	83-07-26
		EP-A-B-	0022019	81-01-07
		FR-A-B-	2459620	81-01-16
		JP-A-	56032488	81-04-01
		JP-B-	62061039	87-12-18
EP-A1- 0274946	88-07-20	DE-A-	3778141	92-05-14
		FR-A-B-	2608051	88-06-17
		JP-A-	63258599	88-10-26
EP-A1- 0065663	82-12-01	JP-A-	57194753	82-11-30
		JP-B-	58054786	83-12-06
EP-A1- 0322589	89-07-05	AU-D-	2659688	89-06-29
		EP-A-	0321603	89-06-28
		JP-A-	2002319	90-01-08
		US-A-	5039532	91-08-13
EP-A1- 0226221	87-06-24	AU-B-	591230	89-11-30
		AU-D-	6834687	87-07-15
		AU-D-	6834787	87-07-15
		EP-A-B-	0250501	88-01-07
		JP-T-	63502003	88-08-11
		JP-T-	63502004	88-08-11
		US-A-	5112812	92-05-12
		WO-A-	87/03785	87-07-02
		WO-A-	87/03786	87-07-02